CHAPTER 5

MORPHOMETRIC AND SEASONAL VARIATIONS IN THE TESTIS
OF CALOTES VERSICOLOR
INTRODUCTION

The pattern of male reproductive cycles in reptiles is known from a large number of lizards and snakes and our knowledge about it prior to 1960 has been reviewed by Harrison Matthews and Marshall (1960). Fitch (1970) has compiled the relevant information on the reproductive cycles in lizards and snakes. The male reproductive cycles in tropical reptiles have not been investigated as extensively as those of temperate region (Fitch, 1970; Kulkarni and Rangaswami, 1971). The seasonal changes in the testis of *H. flaviviridis* has been investigated by Sanyal and Prasad (1965), Prasad and Sanyal, (1966) and Sanyal and Prasad (1967). Similarly, the testicular cycle in *C. versicolor* has been investigated by Sarkar and Rao (1966), Kulkarni and Rangaswami (1972) and Kaminathan and Basu (1973). In the studies on the seasonal changes in the testis of *C. versicolor* there is no mention of Sertoli cells. The Leydig cells of this species have not been adequately described. Kulkarni and Rangaswami (1971) have mentioned that maximum development of Leydig cells coincides with the peak of spermatogenetic activity, maximum epithelial height and the tubule diameter of the epididymis in *C. versicolor*. Extensive phenological studies
correlating the changes in the testicular cycle in reptiles with those of the ambient ecological factors are very few and the reports on this aspect are somewhat sketchy (Fitch, 1970).

Many lizards and snakes are seasonal breeders having a peak of spermatogenic activity occurring once a year either in summer or in winter. In several lizards, especially of the temperate region, the peak of spermatogenic activity takes place during the summer months (Reiss, 1923a, b; Courrier, 1929; Blount, 1929; Herlant, 1933; Cowles and Burleson, 1945; Evans and Clepp, 1940; Breckenridge, 1943). There are also reports that some lizards show two peaks of testicular activity, one during summer and the other during winter (Kohl, 1935; Frankenberger, 1929; Courrier, 1929; Altland, 1941; Cowles and Burleson, 1945). Several lizards exhibit postnuptial spermatogenesis in which proliferation of spermatogonia builds up soon after the breeding is over so that a rapid repopulation of seminiferous tubules with germ cells up to spermatid stage occurs before the winter season (Reiss, 1923a; Herlant, 1933; Regamey, 1935; Altland, 1941; Reynolds, 1943; Cowles and Burleson, 1945; Wilhoft and Quay, 1961). In many vertebrates especially homeothermic forms, the testes are quiescent during winter and the
advanced spermatogenic stages develop during the spring immediately preceding the breeding phase. Thus they show prenuptial type of spermatogenesis (Lofts, 1969).

Most of the studies on reptiles relate to the spermatogenic cycle in lizards and snakes. Some observations have been made on the cyclic variation in the Leydig cells. The Leydig cell cyclicity is observed to correspond often with the spermatogenic activity of the seminiferous epithelium. The maximum activity of the Leydig cells as judged from the abundance of mitochondrial granules and paucity of lipids, has been found to synchronize with the peak of spermatogenic activity as indicated by abundant seminiferous epithelial cells in various stages ranging from spermatogenial cells to spermatozoa in most of the lizards that have been investigated so far. But the situation in the slow-worm, Anguis fragilis is different (Herlant, 1933). In this species, the Leydig cell cycle does not correlate with the spermatogenic activity. On the contrary, its active phase concurs with the active condition of the epididymis and of the sexual segment in the kidneys of males. Presence of similar correlation between the cycle of the Leydig cells and that of male accessory organs and secondary sexual characters has been noticed in a few lizards, A. fragilis
(Heriant, 1933), Lacerta vliglia (Regamey, 1935), C. versicolor (Kasinathan and Basu, 1973). It is surprising to note that the absence of Leydig cells is reported in the testes of Sceloporus undulatus (Altland, 1941) and Lygosoma himalayanum (Koul and Duda, 1976) amongst the large number of lacertilian species that have been examined.

Diverse opinions have been put forward on the spermatogenetic cycle in the testis of C. versicolor. Spermatogenetic activity in the testis of this species is reported to be limited only to the breeding phase (Sarkar and Rao, 1966; Kulkarni and Rangneker, 1971). But according to Kasinathan and Basu (1973) spermatogenetic activity in C. versicolor extends throughout the year but with its peak of activity during the breeding season.

In recent years, considerable attention has been devoted to the study of Sertoli cells of the vertebrate series. These studies mainly envisage the histological and electron microscopic observations and histochemical demonstration of sudanophilia, cholesterol and some important steroid converting enzymes. The ultrastructure of Sertoli cells as revealed by electron microscopic studies is similar to that of steroid producing cells of interrenal and gonads (Lofts, 1968, 1972; Burgos et al., 1970).
As a result of these and other studies, namely, the in vitro conversion of steroids by the isolated seminiferous tubules in rat (Christensen and Mason, 1955) and cobra (Lofts, 1969, 1972; Lofts and Choy, 1971), the steroid biosynthetic ability of Sertoli cells has become increasingly clear. The reptiles in this regard are the least studied and the cyclic activity of Sertoli cells has so far not been investigated. There is no mention of Sertoli cells in the previous studies on the seasonal changes in the testis of *C. versicolor* (Sarkar and Rao, 1966; Kulkarni and Rangnekar, 1971; Kasinathan and Basu, 1973).

Several ambient factors have been considered to be correlated with the breeding cycle in reptiles (Harrison Matthews and Marshall, 1960; Fitch, 1970; Kulkarni and Rangnekar, 1971). Rainfall, temperature, relative humidity, day length and food are some of the major ecological factors that modulate the pattern of reproduction in reptiles. Rainfall seems to be one of the most important ecological factors that influence the testicular cycle in most of the lizards that have been studied (Fitch, 1970). This observation is based on the studies of lizards and snakes belonging to the temperate region. Virtually there is no information available on this aspect in the reptiles of the tropical region which abounds in a large number of reptilian
species. Amongst the several ambient factors such as rainfall, temperature, relative humidity and day length, temperature has been considered to have an effect in bringing the testis of \textit{C. versicolor} into sexual readiness (Kulkarni and Rangnekar, 1971).

In view of the above mentioned facts the present work was undertaken to study in depth, the cyclic changes in the testicular elements such as testis capsule, seminiferous epithelium, interstitial Leydig cells and Sertoli cells in the lizard, \textit{C. versicolor}. Furthermore, an attempt is also made to understand the correlation if any between the seasonal variation in the ecological factors and those of the testicular elements in \textit{C. versicolor} during the reproductive cycle. Incidentally, the results of the present investigations are made use of as the basis for further histochemical studies carried out to elucidate the seasonal changes in the activity of steroid converting enzymes in the testis of \textit{C. versicolor}.

\textbf{MATERIAL AND METHODS}

Adult male specimens of \textit{C. versicolor} were locally collected every month and totally 113 specimens were sacrificed during the course of a year in the present
study. Each month, about 8-11 specimens were weighed and the wet weight of the testes was recorded. A minimum of 5 average looking testes was selected and subjected to morphometric, histological and histochemical investigations.

**Morphometry**

The monthly averages of gonosomatic index were calculated as described in Chapter 2. The diameter of the testes, seminiferous tubules and the height of the seminiferous epithelium were measured from the histological sections by means of an ocular micrometer. The testis diameter was measured from the sections taken from the middle region of the testis. Often the outline of the testis in the sections appeared oblong and only the smallest diameter was recorded from several sections and the average value was calculated from them. For the measurement of the tubule diameter and the epithelial height of the seminiferous tubules, about 10 tubules of large diameter and round in outline, were selected at random from the sections belonging to different specimens of testes. The populations of spermatogonia, primary and secondary spermatocytes and spermatids per unit area of the seminiferous tubules were counted by means of ocular micrograph. The cell countings were made under the oil immersion objective (X98) and the average value of 10 countings was taken into account. The cell and nuclear diameters of
interstitial Leydig cells and the sertoli cells were measured from the histological sections of the testis of *C. versicolor* each month, for a period of one year. All these records are summarised in Table 5.1-5.3 and Graphs 5.1-5.6.

**Histology.** Pieces of testis were fixed in Bouin's fluid for 18 to 20 hours and 5 μm to 7 μm thick paraffin sections were stained with Harris hematoxylin and eosin. Some sections were stained with Mallory's triple stain in order to ascertain the occurrence of different tissues. The histological study of spermatogenetic stages, Leydig cell cycle and the Sertoli cell cycle is based on the observations made on paraffin sections of the testis of *C. versicolor*.

**Ecological factors.** Daily records of maximum and minimum temperature, relative humidity and rainfall for the year were obtained from the Department of Meteorology, College of Agricultural Sciences, Dharwad (Karnataka State, India; 15° 27'N and 75° 3'E) located about 3 kms. away from the laboratory. Monthly averages of maximum and minimum temperature and relative humidity were calculated from the daily records. The mean monthly maximum and minimum temperature is taken as the monthly average temperature while
The records of rainfall denote the actual amount of precipitation that occurred during the month. These records are summarised in Table 2.2 (Chapter 2) and Graphs 5.7, 5.8.

**OBSERVATIONS**

The lizard, *C. versicolor* is a seasonal breeder with the breeding phase occurring during summer months. The testicular cycle showed distinct phases as determined by morphometric and histological studies. The annual testicular cycle may be divided into breeding, regression and regeneration phases.

**Breeding** - During the period from May till the end of August, the values of the gonosomatic index, testis diameter, tubule diameter and the height of the seminiferous epithelium were high (Graph 5.1, 5.2; Table 5.1). The enlarged seminiferous tubules during this period contained cells in all the spermatogenetic stages from actively dividing spermatogonial cells to sperm bundles and sperms (Figs. 5.1, 5.2; Table 5.2). The Sertoli cells were enlarged and did not show well-defined cell boundaries. Only their enlarged nuclei situated along the basement membrane of tunica propria could be discerned. The Leydig
cells were oval with almost round nuclei. These were distinct from the spindle-shaped fibrocytes of the inter-tubular connective tissue. Spermiation judged from the appearance of spermatozoa in the lumen of the seminiferous tubules, began in May (Fig. 5,2) and continued till the end of August (Fig. 5,3). Based on these findings, the span of the breeding phase during the testicular cycle in C. versicolor began in May and extended till August end.

The testis capsule and tunica propria during the breeding period appeared highly thin and distended owing to the increase in the diameter of the testis and seminiferous tubules. There was a small number of fibrocytes in tunica albuginea, tunica propria and the interstitium of the testis. The interstitial area appeared to be decreased and highly vascularised during the breeding season. The breeding activity reached its peak in June when the values for gonosomatic index, testis diameter, tubule diameter and epithelial height of the testis in C. versicolor were the maximum.

**Regression**  - The regression of the testis in C. versicolor began in September and lasted till the end of December. It was characterised by a rapid decrease in the gonosomatic index, shrinkage in the testis diameter,
the tubule diameter and reduction in the epithelial height of the seminiferous tubules (Table 5.1). Only a few spermatogonial cells some of which were exfoliated, were encountered inside the greatly shrunken seminiferous tubules (Fig. 5.4; Graphs 5.3, 5.4; Table 5.2). Sertoli cells at this stage were easily discerned along the parietal layer. Towards the end of regression phase (December), the tubules showed blood plasma and corpuscles interspersed amongst the seminiferous epithelial cells, thus indicating the haemorrhagic condition. Similar feature was also noticed in the interstitial area of the testis in C. versicolor during this phase. By the end of regression period in December, the testis showed the minimum values for gonosomatic index, testis diameter, tubule diameter and the height of seminiferous epithelium (Graph 5.1, 5.2). The tubules that were very much shrunken, appeared solid with thick tunica propria. There were only a few spermatogonial cells in the seminiferous tubules in December (Fig. 5.4) Sertoli cells were small and triangular in shape and they were disposed along the parietal layer. The Leydig cells showed signs of atrophy as judged from vacuolated cytoplasm and the nuclei, both of which did not get stained. Tunica albuginea and tunica propria were thick and vascularised towards the end of regression in C. versicolor.
Regeneration phase of the testis in *C. versicolor* was marked by a gradual increase in the gonosomal index, enlargement of testis diameter and the tubule diameter in the month of January (Table 5.1; Graphs, 5.1, 5.2; Figs. 5.5, 5.6). During the beginning of regeneration, there was an increase in the spermatogonial cells showing cell division. Thus, there was an overall increase in the seminiferous epithelial cells and various stages of spermatogenesis from spermatogonia to secondary spermatocytes were noticed towards the middle of the regeneration period (February). The spermatogenic stages from spermatogonia to spermatids were seen in the terminal period of regeneration. The formation of sperm and their release seemed to coincide with the onset of the breeding season. There was also an increase in the number of Leydig cells concomitant with the increase in the number of fibrocytes in the interstitium.

**Leydig cell cycle:** The Leydig cells in the testis of *C. versicolor* show morphological changes depending upon the phases of reproductive cycle (Graph 5.3; Table 5.3). They seem to arise during the onset of regeneration. To start with, they are spindle-shaped closely resembling the fibrocytes of the interstitial connective tissue but larger in size. During the breeding phase, they were large and
ovoid in shape. The Leydig cells belonging to the regression phase were atrophied and they showed signs of involution.

Sertoli cell cycle: The cyclic changes in the morphology of Sertoli cells of *C. versicolor* correlated closely with those of other testicular elements. An increase in the cell size was noticed during the latter half of the regeneration phase when spermatocytes and spermatids began to appear in the seminiferous tubules (Graph 5.6; Table 5.3). At this time the outlines of these cells became obscured probably due to the development of cellular extensions. In addition, the outline of the Sertoli cells was not easily made out during the breeding phase, since the tubules were compactly packed with a large number of cells ranging from spermatogonia to spermatids, sperm bundles and sperms. However, their occurrence could be ascertained from their nuclei situated along the basement membrane of tunic propria. Sertoli cells of the regression phase were small triangular or triradiate or subcolumnar cells with their extended base lining the basement membrane of tunica propria of the seminiferous tubules (Fig. 5.7).

Seasonal changes: The seasonal changes in the ambient atmospheric conditions correlated well with the changes in the testicular elements in *C. versicolor* during
the annual cycle. Monthly records of temperature during the year showed that there was an increase in average temperature from January till the month of April when it reached its summer maximum. It showed a downward trend from May till December after registering a slight increase during September and October (Graph 5.7). Incidentally, the difference between the maximum and minimum temperature during the period from May to August was lower than that of other months of the year (i.e., September to April).

Relative humidity was at its ebb in January and it gradually increased though insignificantly, from then onwards till March. There was a rapid rise in relative humidity from May till it reached its maximum in August. It recorded from August till the end of December (Graph 2.1). A few spells of monsoon showers, at times heavy but of short duration, occurred in March till the end of May. The incessant drizzles of longer duration started only in June and continued till the end of August (Graph 2.2). The period of September and October witnessed a few squalls of heavy precipitation of short duration. The geographical location of Dehraudin is such that it experiences the shortest day in January and the longest in July. From January to July the day length gradually increases and it decreases from July till January.
The brooding phase of male *C. versicolor* occurred when the monthly average temperature and relative humidity were high and the difference between the maximum and minimum temperature was less during May-August. The relative humidity during this period approximately ranged between 80% and 90%. The breeding period, May - August, also witnessed continuous rains and longer days. The regression of the testis in *C. versicolor* ensuing between September - December coincided with the decrease in average temperature, increase in the difference between maximum and minimum temperature and decrease in relative humidity and day length. The regeneration of the testis occurred when there was a rise in temperature and day length.

**DISCUSSION**

The present study showed that the lizard, *C. versicolor* is a seasonal breeder, the breeding phase of which synchronizes with the summer months, May to August. It breeds only once during the annual cycle and the peak of spermatogenetic activity as judged from the records of high gonosomal index and maximum cell population of spermatogenesis, occurs in June. The onset of regeneration in January is characterized by an increase in the
number of spermatogonial cells that fill the lumen of
the shrunken seminiferous tubules so much so that the
tubules appear almost solid. The advanced stages of
spermatogenesis showing germ cells ranging from sperma-
togonia to spermatids are seen till the end of April which
marks the end of regeneration phase. Thus C. versicolor
shows the prenuptial type of spermatogenesis. Several
studies have shown that the spermatogenetic cycle is
limited to the breeding phase that occurs during the
summer months in the lizards, Lacerta muralis, L. vigilis
(Reiss, 1923a,b), Phrynosoma solare (Blount, 1929), L.
vivipara, Chalcides ocellatus, Hemidactylus mabuya
(Herlant, 1933), Anolis carolinensis (Evans and Clapp, 1940),
Surnesia septentrionalis (Breckenridge, 1943), Xantusia
vigilis (Cowles and Burleson, 1945), Hemidactylus
flaviviridis (Sanyal and Prasad, 1965, 1967; Prasad and
Sanyal, 1966) and C. versicolor (Sarker and Rao, 1966;
Kulkarni and Rangnakor, 1971). In the tropical forms such
as Lacerta agilis (Frankenberger, 1928), Acanthodactylus
pardalis (Kohl, 1935) and Scleropus undulatus (Altland,
1941) the peak of testicular activity is seen in winter. In
L. agilis (Frankenberger, 1928), Uromastix sp. (Courrier,
1929) and X. vigilis (Cowles and Burleson, 1945), two peaks
of spermatogenetic activity, one in summer and the other in
winter are noticed. Thus they exhibit postnuptial spermatogenesis. According to Kesinathan and Basu (1973), spermatogenesis in *C. versicolor* is seen throughout the testicular cycle with its maximum activity synchronising with the summer months. However, our observations and also those of other workers (Sarkar and Hoo, 1966; Kulkarni and Rannangker, 1971) establish that spermatogonetic cycle starts immediately after winter in *C. versicolor* and spermatids are developed towards the end of April. Thus our studies on the seasonal changes in the testis of *C. versicolor* shows that the spermatogenesis in this species is of postnuptial type in which the spermatogonatic activity starts only after the winter is over, a fact which was not reported by earlier workers. In many tropical reptiles showing spermatogonotic activity immediately after breeding, spermatogenesis is of postnuptial type (Lofts, 1968).

In fishes and amphibians that show cystic spermatogenesis, the sertoli cells are known to arise de novo from the fibroblasts of tunica propria during the period of regeneration while they form a permanent feature in reptiles, birds and mammals (Lofts, 1968). They arise from the sex chord during the development and their number inside the seminiferous tubules remains fixed bearing a definite ratio
to spermatoocytes and spermatids in amniotes (Lofts, 1968, 1972; Courrot et al., 1970). Sertoli cells in amniotes which show noncystic spermatogenesis, extend their cytoplasmic processes to establish contact with spermatoocytes and spermatids. In fishes and amphibians showing cystic spermatogenesis, the Sertoli cells form the cyst or capsule that holds the spermatoocytes, sperm and sperm bundles (Lofts, 1968). A new generation arises during the resumption of the next cycle in the anamniotes that exhibit cystic spermatogenesis. Sertoli cells of reptiles in this respect are the least investigated. In the studies on the testicular cycle in *C. versicolor* (Serbar and Rao, 1966; Kulkarni and Rangmacher, 1971; Kesinathan and Basu, 1973) Sertoli cells and their cycle are not investigated. Our histological observations on the cyclic changes in the testis of *C. versicolor* reveal that the Sertoli cells undergo seasonal morphological changes. The fact that neither they detach from the basement membrane of tunica propria nor do they show mitotic figures during the different phases of the reproductive cycle, may suggest that they are the permanent feature inside the seminiferous tubules as in other amniotes. However further work is needed to verify whether the number of Sertoli cells remains fixed or not in *C. versicolor*. 
The interstitial Leydig cells in the testis of many reptiles have been observed to undergo cyclic morphological changes (Hiller, 1950; Harrison Matthews and Marshall, 1960; Lofts, 1968, 1969). The enlarged Leydig cells in the testes of *L. muralis* and *L. agilis* occur synchronously with the growth and activity of the secondary sexual characters (Reiss, 1923a,b). The Leydig cell activity in the testis of *L. agilis* has been investigated by Frankenberger (1923) and later by Regamey (1935). According to Frankenberger (1923), there are two maximal periods of Leydig cell development which correspond with two peaks of spermatogetic activity in this species while Regamey (1935) noticed the development of male accessory organs and secondary sexual characters synchronising with the development of Leydig cells. In a few lizards such as the grass snake, *Natrix natrix* (Herlant, 1933), the musk turtle, *Stenotherus odoratus* (Risley, 1938), *X. vigillia* (Miller, 1948) and *Natrix piscator* (Srivasstava and Thapliyal, 1965) the interstitial cells lack demonstrable seasonal changes in number, size and activity.

Sarkar and Rao (1966) and Kasinathan and Basu (1973) who studied the seasonal changes during the testicular cycle in *C. versicolor* have not investigated the Leydig cell cycle in this lizard. Kulkami and Angmekker (1971)
have measured the nuclear diameter of the Leydig cells of *C. versicolor* in their studies on the seasonal changes in the testis of this lizard and according to them the measurement of the nuclear diameter was possible only during the months of June, July and October to December. They, however, claim that the Leydig cells undergo morphological changes that correlate with spermatogenesis and the epithelial height of epididymis. Our observations show that the Leydig cells of *C. versicolor* undergo seasonal changes in their morphometry. The increase in their diameter observed during the breeding months from May till the end of August might reflect their increased activity during the breeding phase.

Pitch (1970) has analysed the data from the previous literature on the timing of the breeding season in lizards and snakes. According to him, geographic distribution of the species and the climatic conditions are the determining factors which influence the reproductive cycles in lizards. In the cooler parts of the temperate region, the reptiles invariably exhibit a short and well-defined breeding season that ensues in spring. In the mid-temperate zone, the breeding season is of longer duration. In several lizards of the arid or xeric conditions such
as *Z. vigilia, Uma inornata, U. scoparia, Uta stansburiana*
and *Scaloporus occidentalis*, the breeding phase is demonstrably
correlated with the amount and duration of rainfall,
presumably through their effect on the vegetation and on
the insect populations. The reproductive cycle may be
partly or wholly suppressed in years of paucity of rains
or inadequate precipitation. In warm temperate and subtrop-
ical regions breeding season tends to be lengthened and
it is timed more with seasonal distribution of rainfall
than with the annual temperature cycle. Majority of the
tropical and subtropical lizards such as geckos, anoles,
agamids, chameleons, skinks and lacertids display extended
breeding season which in some instances lasts throughout
the year.

In the neotropical (South American) lizard, *Anolis
carolinensis* spermatogenesis is initiated in autumn and it
is influenced by decreasing day length (Fox and Dessauer,
1958). In *Crotaphytis wislizenii* breeding phase is corre-
lated with heavy rains followed by luxuriant growth of
vegetation and increase in insect populations. Conversely,
poor reproduction or its total failure occurred during the
years of scant rains or total drought (Turner et al., 1969).
Similar observations are made in the study on seasonal
cycle in *Agama agama* (Loveridge, 1936; Chapman and Chapman,
Experimental studies on *X. vulgaris* showed that the males maintained at higher temperatures showed accelerated gonadal development (Bartholomew, 1953). Kulkarni and Rangnaker (1971) who have studied the testicular changes in relation to climatic conditions, have noted that the cyclic changes in the testis of *G. versicolor* are correlated to temperature. Testicular atrophy similar to regression of the testis during the winter months was noticed in *Thamnophis* maintained below 20°C during the breeding months and the onset of spermatogenesis coincided with the vernal rise in temperature (Fox, 1954). Furthermore, a number of reports claim that high temperature has a stimulatory effect on spermatogenesis at the same time reducing the responsiveness of the Leydig cells to synthesize androgens (van Oordt, 1963; Licht, 1972). It is not known whether the action of high temperature is direct on the steroidogenic cells or is mediated through the lowered gonadotropin levels (Licht, 1972).

Thus in the attempts to establish the correlation between the cyclic changes in the testicular elements of reptiles and those of ambient factors, rainfall, temperature, day length and relative humidity have been considered to act upon the breeding activity in reptiles. So far, these researchers tend to indicate that rainfall and temperature...
are the foremost amongst the ecological factors. Our observations show that *C. versicolor* is a tropical lizard with a distinct seasonal cycle. The spermatogenetic activity of this lizard is limited to the regeneration and breeding periods that extend from January till the end of August, contrary to the observation (Kasinathan and Basu, 1973) that it extends throughout the annual cycle. Furthermore, our study on the testicular cycle of *C. versicolor* indicates that the breeding activity concurs with the (1) rise in monthly average of maximum and minimum temperature, (2) decrease in the difference between maximum and minimum temperature, (3) rise in relative humidity and (4) day length and (5) monsoon rains. Our studies do not permit us to evaluate the isolated effect or the relative influence of any of the individual factors on the testicular changes during the annual cycle of this lizard.

**SUMMARY**

1. The histomeric study of gonosomatic index testis diameter, seminiferous tubule diameter, height of the seminiferous epithelium and the histological changes in the testis of *C. versicolor* carried out each month for one year, show that *C. versicolor*
is a seasonal breeder showing well-defined phases, namely, regeneration (January - April), breeding (May - August) and regression (September - December).

2 The testis capsule, seminiferous tubules and the interstitium undergo cyclic changes.

3 Spermatogenic activity starts in January that marks the beginning of regeneration and spermatids are developed by April. Spermiation occurs in May when breeding begins.

4 The testis enters into regression in September and its quiescent stage continues till December. During this phase a few spermatogonial cells and small-sized Sertoli cells are found inside highly shrunken seminiferous tubules.

5 Monthly observations show that the Leydig cells and Sertoli cells undergo morphological changes that coincide with the phases of the testicular cycle.

6 Spermatogenesis in *C. versicolor* is of noncystic and pronuptial type.

7 The histometric changes during the testicular cycle correspond with the seasonal fluctuations.
in temperature, relative humidity, day length and rainfall.

Breeding occurs when temperature is high, the difference in maximum and minimum temperature is low, the days are long and there is an increased humidity and widespread rains;
Table 5.1 Monthly records of gonosomatic index, testis diameter, tubule diameter and the height of seminiferous epithelium in the testis of C. varicolor.

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of males</th>
<th>Gonosomatic index</th>
<th>Testis diameter</th>
<th>Tubule diameter</th>
<th>Epithelial height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>8</td>
<td>0.5232 ± 0.00253*</td>
<td>1690 ± 5.676*</td>
<td>75.62 ± 0.735*</td>
<td>35.72 ± 0.6306*</td>
</tr>
<tr>
<td>Feb</td>
<td>10</td>
<td>0.1372 ± 0.007244</td>
<td>2190 ± 14.18</td>
<td>115.52 ± 1.213</td>
<td>49.40 ± 0.0312</td>
</tr>
<tr>
<td>Mar</td>
<td>10</td>
<td>0.5403 ± 0.00672</td>
<td>4140 ± 13.50</td>
<td>197.52 ± 1.989</td>
<td>61.18 ± 0.6811</td>
</tr>
<tr>
<td>Apr</td>
<td>11</td>
<td>0.6154 ± 0.0021</td>
<td>1670 ± 6.75</td>
<td>69.54 ± 0.7251</td>
<td>22.42 ± 0.2803</td>
</tr>
<tr>
<td>May</td>
<td>10</td>
<td>2.3589 ± 0.03977</td>
<td>4390 ± 24.02</td>
<td>223.9 ± 1.865</td>
<td>70.18 ± 0.8455</td>
</tr>
<tr>
<td>Jun</td>
<td>10</td>
<td>2.933 ± 0.03064</td>
<td>5170 ± 44.98</td>
<td>298.0 ± 6.087</td>
<td>68.38 ± 0.6826</td>
</tr>
<tr>
<td>Jul</td>
<td>10</td>
<td>2.1077 ± 0.05109</td>
<td>3040 ± 14.33</td>
<td>163.54 ± 2.517</td>
<td>30.14 ± 0.7625</td>
</tr>
<tr>
<td>Aug</td>
<td>8</td>
<td>1.4250 ± 0.1350</td>
<td>3440 ± 20.11</td>
<td>153.60 ± 1.622</td>
<td>35.68 ± 1.002</td>
</tr>
<tr>
<td>Sept</td>
<td>8</td>
<td>0.2644 ± 0.00118</td>
<td>1660 ± 6.171</td>
<td>79.88 ± 0.8271</td>
<td>24.72 ± 0.4746</td>
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<tr>
<td>Oct</td>
<td>10</td>
<td>0.1156 ± 0.00218</td>
<td>1680 ± 7.543</td>
<td>73.16 ± 0.8389</td>
<td>18.28 ± 0.4821</td>
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<tr>
<td>Nov</td>
<td>10</td>
<td>0.2932 ± 0.00018</td>
<td>1640 ± 4.800</td>
<td>86.60 ± 0.8153</td>
<td>31.16 ± 0.4671</td>
</tr>
<tr>
<td>Dec</td>
<td>8</td>
<td>0.05602 ± 0.00005</td>
<td>1170 ± 9.486</td>
<td>74.02 ± 0.8598</td>
<td>30.02 ± 0.2803</td>
</tr>
</tbody>
</table>

( * Denotes the standard error of the mean records)
Table 5.2 - Monthly averages of the number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids in the testis of G. versicolor.

<table>
<thead>
<tr>
<th>Months</th>
<th>Spermatogonia</th>
<th>Primary Spermatocytes</th>
<th>Secondary Spermatocytes</th>
<th>Spermatids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>75 ± 10.4</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Feb</td>
<td>59.4 ± 12.33</td>
<td>23.4 ± 10.47</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Mar</td>
<td>51.4 ± 12.29</td>
<td>53.6 ± 11.80</td>
<td>43.6 ± 13.71</td>
<td>62.2 ± 22.23</td>
</tr>
<tr>
<td>Apr</td>
<td>40.6 ± 9.33</td>
<td>62.8 ± 14.66</td>
<td>49.2 ± 15.09</td>
<td>90.7 ± 29.34</td>
</tr>
<tr>
<td>May</td>
<td>29.9 ± 6.36</td>
<td>72.00 ± 17.52</td>
<td>54.8 ± 13.47</td>
<td>119.2 ± 36.45</td>
</tr>
<tr>
<td>Jun</td>
<td>34.4 ± 6.7</td>
<td>53.8 ± 15.01</td>
<td>50.9 ± 20.04</td>
<td>105.8 ± 37.2</td>
</tr>
<tr>
<td>Jul</td>
<td>19.2 ± 2.95</td>
<td>55.6 ± 25.3</td>
<td>35.0 ± 16.97</td>
<td>105.8 ± 7.19</td>
</tr>
<tr>
<td>Aug</td>
<td>44.8 ± 3.59</td>
<td>27.6 ± 12.83</td>
<td>16.6 ± 7.5</td>
<td>74.4 ± 22.74</td>
</tr>
<tr>
<td>Sep</td>
<td>33.4 ± 6.86</td>
<td>24.8 ± 9.47</td>
<td>N11</td>
<td>N11</td>
</tr>
<tr>
<td>Oct</td>
<td>22.0 ± 10.13</td>
<td>22.0 ± 6.12</td>
<td>N11</td>
<td>N11</td>
</tr>
<tr>
<td>Nov</td>
<td>68.8 ± 14.14</td>
<td>N11</td>
<td>N11</td>
<td>N11</td>
</tr>
<tr>
<td>Dec</td>
<td>21.00 ± 2.84</td>
<td>N11</td>
<td>N11</td>
<td>N11</td>
</tr>
</tbody>
</table>

(*) Denote the values of the standard error of the averages)
Table 5.3 - Monthly records of cell diameter and nuclear diameter of the Leydig cells and Sertoli cells in the testis of *C. versicolor.*

| Months | **Leydig cells** | | **Sertoli cells** | |
|--------|------------------|------------------|
|        | Cell diameter    | Nuclear diameter | Cell diameter    | Nuclear diameter |
| Jan    | 4.6 ± 0.8          | 1.4 ± 0.00       | 4.7 ± 0.33       | 1.4 ± 0.00       |
| Feb    | 6.4 ± 1.52         | 1.9 ± 0.26       | 5.2 ± 1.12       | 1.8 ± 0.31       |
| Mar    | 7.1 ± 1.43         | 1.7 ± 0.33       | 6.7 ± 1.17       | 1.4 ± 0.00       |
| Apr    | 7.45 ± 0.90        | 1.6 ± 0.29       | 7.2 ± 1.24       | 1.4 ± 0.00       |
| May    | 7.9 ± 0.37         | 1.5 ± 0.25       | 7.6 ± 1.32       | 1.4 ± 0.00       |
| Jun    | 7.4 ± 1.09         | 1.9 ± 0.94       | 9.1 ± 0.82       | 3.6 ± 0.76       |
| Jul    | 9.9 ± 1.28         | 4.9 ± 0.70       | **-** ± **-**     | 8.2 ± 1.02       |
| Aug    | 11.4 ± 1.41        | 3.8 ± 0.42       | **-** ± **-**     | 7.8 ± 0.77       |
| Sept   | 6.6 ± 0.70         | 2.5 ± 0.28       | 5.5 ± 1.10       | 4.6 ± 0.38       |
| Oct    | 5.6 ± 0.00         | 1.4 ± 0.00       | 4.7 ± 1.25       | 1.4 ± 0.00       |
| Nov    | 5.0 ± 0.99         | 1.4 ± 0.00       | 5.4 ± 0.31       | 1.4 ± 0.00       |
| Dec    | 4.00 ± 0.97        | 1.4 ± 0.00       | 4.9 ± 0.62       | 1.4 ± 0.00       |

(* Denotes the standard error of the mean records)
(** No records could be made as cell outlines were not clearly seen)
EXPLANATION TO FIGURES

Graph 5.1  Monthly records of gonosomal index (GI) and the testis diameter (TD) of C. versicolor.

Graph 5.2  Monthly records of seminiferous tubule diameter (TUD) and the height of seminiferous epithelium (SH) in the testis of C. versicolor.
EXPLANATION TO FIGURES

Graph 5.3  Monthly records of the number of spermatogonia (SPG) and primary spermatocytes (SPC (I)) per unit area in the testis of C. versicolor.

Graph 5.4  Records of numbers of secondary spermatocytes (SPC (II)) and spermatids (SPT) per unit area made every month from the testis of C. versicolor.

Graph 5.5  Monthly variation in the diameters of the Leydig cells (LCD) and their nuclei (IND) in C. versicolor.

Graph 5.6  Monthly variation in the diameters of the Sertoli cells (SCD) and their nuclei (SND).
EXPLANATION TO FIGURES

Graph 5.7  Monthly records of maximum, minimum and mean temperature and relative humidity.

Graph 5.8  Histogram showing the actual amount of rainfall during the months of the annual cycle.
EXPLANATION TO FIGURES

Fig. 5.1 Section of the testis of *C. versicolor* during the onset of breeding (May). The seminiferous tubules are filled with cells in different stages of spermatogenesis. X400.

Fig. 5.2 Section of the testis of *C. versicolor* during the middle of the breeding season (July). X200.

Fig. 5.3 Section of the testis of *C. versicolor* towards the end of the breeding season. Some tubules are devoid of sperms indicating that spermiation has taken place. X100.

Fig. 5.4 Section of the testis of *C. versicolor* towards the end of regression (December). The seminiferous tubules appear shrunken and disorganised. X100.
Section of the testis during onset of regeneration (January). The interstitial area shows marked increase in the connective tissue cells and the number of spermatogonial cells begins to increase. X100.

Section of the testis during the middle of the regeneration phase (March). The seminiferous tubules appear solid and are replete with spermatogonial cells. X100.